Article

Assessment of commercial probiotic bacterial contents and label accuracy

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Abstract — Probiotics are widely available for use in animals but quality control of veterinary probiotics has been shown to be poor. The objective of this study was to evaluate the labels and bacterial contents of commercial probiotics marketed for use in animals. Twenty-five probiotics were purchased, labels were scrutinized, and bacterial contents were enumerated. Twenty-one (84%) products listed specific microorganisms. Expected bacterial numbers were listed for 15 (60%) products. One or more organisms were misspelled on the labels of 7/22 (32%) of products that listed specific organisms. Viable growth ranged from 0 to 2×10^9 colony forming units (CFU)/g. Only 4/15 (27%) products that had specific claims of viable organisms met or exceeded their label claim. Only 2 of these also had an acceptable label, which properly described the contents. Deficiencies in veterinary probiotic quality remain. Veterinarians and owners should scrutinize commercial probiotics and demand evidence of quality control and efficacy.

Résumé — Évaluation du contenu bactérien probiotique commercial et de l'exactitude des étiquettes. Les probiotiques sont généralement disponibles pour usage chez les animaux mais le contrôle de la qualité des probiotiques vétérinaires s'est avéré médiocre. L'objectif de cette étude consistait à évaluer les étiquettes et le contenu bactérien des probiotiques commerciaux commercialisés pour usage chez les animaux. Vingt-cinq probiotiques ont été achetés et les étiquettes ont été minutieusement examinées et le contenu bactérien a été énuméré. Vingt et un produits (84 %) énuméraient des microorganismes particuliers. Les numérations bactériennes prévues ont été énumérées pour 15 produits (60 %). Le nom d'un ou de plusieurs organismes était mal orthographié sur les étiquettes de 7/22 des produits (32 %) qui énuméraient des organismes particuliers. La croissance viable s'échelonnait de 0 à 2×10^9 de cellules souches unipotentes (CFU)/g. Seulement 4/15 produits (27 %) qui affichaient des allégations spécifiques d'organismes viables satisfaisaient aux allégations de l'étiquette ou les dépassaient. Seulement 2 de ces produits possédaient aussi une étiquette acceptable qui décrivait adéquatement le contenu. Des lacunes au niveau de la qualité des probiotiques vétérinaires sont toujours présentes. Les vétérinaires et les propriétaires devraient examiner les probiotiques commerciaux et exiger des preuves du contrôle de la qualité et de l'efficacité.

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Probiotics are live microorganisms, that when ingested at certain doses, provide a health benefit beyond that of their inherent nutritional value (1). Probiotics are an appealing approach for prevention or treatment of many conditions in animals because of the potential to be effective and safe, and to reduce the use of antimicrobials. Various probiotics are available commercially for use in veterinary medicine, although published clinical efficacy data are lacking. Despite this, probiotics are widely used in veterinary practice and by pet owners. Because probiotics are nutraceuticals, not pharmaceuticals, there is minimal regulatory scrutiny. Previous studies have identified discrepancies between stated and actual contents in commercial products for both human and veterinary use (2–4). In one study of veterinary probiotics, over half of the tested veterinary probiotics did not specifically list their contents and most contained very low levels of viable organisms (4). Another study that scrutinized probiotics labels demonstrated other deficiencies, including frequent misidentification of bacteria and misspelling of contents (5).

Given the increased attention to the quality of probiotics and other commercial nutraceuticals and the release of new commercial products, it is possible that the quality of available probiotics has improved. The objective of this study was to evaluate labels and bacterial contents of commercial veterinary probiotics.

Materials and methods

Probiotics were purchased at retail outlets in Ontario, Canada and through Internet sites. When more than 1 product was

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available from a specific source, all products were purchased to avoid selection bias.

Product labels were scrutinized; this included an assessment of spelling of contents, if contents were clearly described (genus and species), if the number of viable organisms was stated, if there was an expiry date, and if specific health claims were made.

The probiotic (1 g) was added to 9 mL phosphate-buffered saline (PBS) and vortexed thoroughly. Serial 10-fold dilutions were then made in PBS. A 100-μL volume of each dilution was inoculated onto deMan, Rogosa, Sharpe (MRS) agar, and 2 Columbia blood agar plates. The MRS plates were incubated anaerobically at 37°C for 48 h for isolation of lactic acid bacteria. Lactic acid bacteria were identified by Gram stain, catalase reaction, and colony morphology. Blood agar plates were incubated aerobically at 35°C for identification of enterococci, streptococci, and bacilli, and anaerobically at 37°C for identification of bacilli. Enterococci and streptococci were identified by colony morphology on blood agar, Gram stain appearance, and catalase reaction. *Bacillus* spp. were identified by Gram stain, colony morphology, and biochemical characteristics. The 1 exception was a product that was in a vegetable oil-based chewable format. Based on manufacturer's recommendations, the product was added to PBS in a sterile bag and incubated at 35°C for 10 min before massaging the tablet manually to dissolve it. Serial dilutions were then performed.

Colony counts were recorded. In situations where more than one similarly appearing organism was listed on the label (multiple *Lactobacillus* spp.), no attempt was made to assess the numbers of different organisms because of the difficulties in accurately quantifying different species. All testing was performed in triplicate.

Descriptive statistics were applied. The percentage of detected bacterial growth compared to the label claim was calculated by using the following formula:

$$\text{Actual count (CFU/g)/Label claim (CFU/g)} \times 100$$

Results

Twenty-five products (Table 1) were evaluated; 16 (64%) were powders, 7 (28%) were in capsules, 1 was a chewable formulation, and 1 was a liquid. Twenty-one (84%) products listed specific microorganisms, ranging from 1 to 11 different species per product (median 4). "Bacterial culture," "probiotics," or "probiotic blend" were listed by 3 products, while 1 product claimed to contain *Bacillus subtilis* and "mixed lactic acid bacteria." Expected bacterial numbers were listed for only 15 (60%) products. Conflicting information was given for 1 product, with a table stating that 600 colony forming units (CFU)/g of *Bacillus coagulans* were present and a footnote stating "*Lactospore sporogenes* = *Bacillus coagulans* 100 million/gram." Another product stated that 60 mg of probiotics were included, with no indication of actual numbers. Similarly, 1 product claimed there were 600 mg/3 capsules, with no indication of how that corresponded to numbers of viable bacteria. The remaining products made no mention whatsoever of the expected number of organisms. All but 1 product listed an expiry date. Three products claimed to contain "*Lactospore sporogenes*" or "*Lactobacillus sporogenes*," organisms that do not exist but names that are sometimes used by probiotic manufacturers to describe *Bacillus coagulans*. All 3 products did contain a notation that the organism is also known as *B. coagulans*. Misspelling was a problem, with ≥ 1 organisms misspelled on the labels of 7/22 (32%) products that listed specific organisms.

If an adequate label is defined as one that contains specific (and valid) bacterial names (genus and species), with no spelling errors and a clear statement of number of viable organisms that are expected, then only 8 (32%) products were properly labeled. If a more stringent definition of an adequate label is used, which includes a requirement that the product identify the specific bacterial strain that is present, then no products were adequately labeled.

Viable growth ranged from 0 to 2×10^9 CFU/g (Table 1). The product with no growth claimed to contain *Lactobacillus acidophilus* yet had no growth on all 3 replicates. Testing was repeated using different capsules from the same bottle and the absence of growth was confirmed. Only 4/15 (27%) products that had specific claims of viable organisms met or exceeded their label claim. Only 2 of these also had an acceptable label, which properly described the contents, meaning only 2/25 (8%) products in this study had both an acceptable label and viable bacterial growth that met or exceeded label claims.

Discussion

Numerous product deficiencies were identified in this study, particularly inadequate description of contents (organisms and numbers), misspelling of bacteria and low bacterial numbers (both compared to label claims and overall). Only 2 of the 25 evaluated products were considered acceptable when both the label and contents are considered, something that is of great concern. While direct comparison to the earlier study of veterinary probiotics (4) should be avoided because of different products and methodology, it is clear that serious product quality issues remain.

The overall level of bacterial growth was highly variable. It is unclear whether the products with low levels actually had the stated numbers at the time of manufacturing or whether the stated levels were never present. Regardless of whether or not the organisms died during storage or were never there is irrelevant for the patients. One product had no viable growth, despite claiming to contain 14 million CFU/capsule. This product was, as for all others, tested before its expiry date and was not handled differently than any other products.

It was somewhat encouraging that many products had reasonably high numbers of viable bacteria, even though levels were typically less than the label claim. Eight (32%) products contained $>$ 100 million CFU/g. The clinical relevance of these (or even higher) levels is unclear, however, because of the lack of information about doses required for different organisms. At the time of writing, no published studies had demonstrated a positive health effect for any commercial probiotic for companion animals. Thus, it is unclear if any of these products would be effective at any dose or whether different doses would suffice for different organisms. Colonization and clinical studies involving dogs and cats, which tended to use empirical dosing, have

Table 1. Bacterial contents and label evaluation for 25 commercial veterinary probiotics

Probiotic	Label organisms	Actual concentration[a] (CFU/g)	% of claim[b]	Correct spelling	Adequate label	Adequate label and contents
A	*Bifidobacterium animalis*	2×10^9	920	Yes	Yes	Yes
B	Bacterial culture	2×10^7	NA	NA	No	No
C	Mixed lactic acid bacteria, *Bacillus subtillus* (sic)	1.7×10^4	0.9	No	No	No
D	*L. acidophilus, L. casei, Enterococcus faecium, B. subtilus* (sic), *B. licheniformis, B. coagulins* (sic)	1.1×10^8	0.9	No	No	No
E	*L. acidophilus, L. casei, Enterococcus faecium, B. subtilus* (sic), *B. licheniformis, B. coagulins* (sic)	2.3×10^6	1.8	No	No	No
F	*Bacillus subtilis, Bacillus lichenformis, Lactobacillus*	7.2×10^7	NA	Yes	No	No
G	*L. acidophilus, L. plantarum, L. brevis, L. rhamnosus, B. bifidus* (sic), *B. longum*	4.8×10^5	NA	No	No	No
H	*L. acidophilus, B. thermophilum, B. longum, Enterococcus faecium*	2.7×10^7	2.7	Yes	Yes	No
I	*Bacillus coagulans (L. sporogenes)*	4×10^5	NA	Yes	No	No
J	Probiotics	1×10^4	NA	NA	No	No
K	*Lactobacillus acidophilus, Bifidobacterium longum, B. bifidum, B. infantis*	1.6×10^7	0.16	Yes	Yes	No
L	Probiotic blend (6 bioactive strains)	1×10^6	2.3	NA	No	No
M	*Bacillus subtillis* (sic), mixed lactic acid bacteria	2.3×10^6	3194	No	No	No
N	*Enterococcus faecium*	2.6×10^8	260	Yes	Yes	Yes
O	*E. thermophilus, L. acidophilus*	5.7×10^8	NA	Yes	No	No
P	*L. acidophilus, Bifidobacterium bifidum, B. infantis, B. longum, L. bulgaricus, L. casei, L. salivarius, S. thermophilus*	4×10^8	16	Yes	Yes	No
Q	*Lactobacillus sporogenes*	1×10^9	247	Yes	No	No
R	*Bacillus coagulans (L. sporogenes), Bacillus subtilis*	6×10^6	NA	Yes	No	No
S	*L. acidophilus, B. bifidum, B. infantis, B. longum, L. helveticus, L. casei, L. salivarius, S. thermophilus*	3×10^5	NA	Yes	No	No
T	*L. acidophilus, B. bifidum, B. infantis, B. longum, L. helveticus, L. casei, L. salivarius*	9×10^8	NA	Yes	No	No
U	*L. acidophilus*	0	0	Yes	Yes	No
V	*L. acidophilus, B. longum*	3.6×10^9	50	Yes	Yes	No
V	*B. bifidum, L. lactic* (sic), *L. acidophilus*	1×10^7	7	No	No	No
X	*L. acidophilus, B. bifidum, B. infantis, B. longum, L. casei, L. helveticus, L. plantarum, L. rhamnosus, L. salivarius, L. lactis*	3×10^8	38	Yes	Yes	No
Y	*Lactobacillus paracasei, L. curvatus, L. rhamnosis* (sic), *L. plantarum*	4.5×10^5	NA	No	No	No

A — Prostora Max, The Iams Company, Cincinnati, Ohio 45202, USA; B — Digest-Aide, Canine Herbals, Guelph, Ontario N1H 1E9; C — digest-aide, McIntosh ProLine Products Inc, Wheatley, Ontario N0P 2P0; D — Holistic Solutions, Eagle Pack Pet Foods Inc, Mishawaka, Indiana 46544, USA; E — Holistic Transitions, Eagle Pack Pet Foods Inc, Mishawaka, Indiana 46544, USA; F — PROBIOplus, Herbs for Horses, Guelph, Ontario N1H 1E9; G — Equine Biotic 8, Omega Alpha Pharmaceuticals Inc, Toronto, Ontario M1L 3K2; H — N'Zymes, Biopet Inc, Las Vegas, Nevada 89193-4347, USA; I — Geneflora, Cycles of Life, Newbury Park, California 91320, USA; J — Seagreens Powder, Holistic Blend, Mississauga, Ontario L5L 6A6; K — Rx Biotic, Rx Vitamins Inc, Elmsford, New York 10523, USA; L — Rx Nutrigest, Rx Vitamins Inc, Elmsford, New York 10523, USA; M — Acute Care Supportive G.I. Powder, Centaur VA Animal Health, Guelph, Ontario N1H 6T9; N — FortiFlora, Nestle Purina PetCare Company, Mississauga, Ontario L5J 1K7, USA; O — Azodyl, Vétoquinol Canada Inc, Lavaltrie, Quebec J5T 3S5; P — Probiotic Blend, Only Natural Pet Store, Boulder, Colorado 80301, USA; Q — *Lactobacillus sporogenes,* Thorne Research, Dover, Indiana 83825, USA; R — Plant Enzymes & Probiotics, Animal Essentials, Victor, Montana 59875, USA; S — Spectra Probiotic, Integrative Therapeutics Inc, Green Bay, Wisconsin 54311, USA; T — Blue Heron, Integrative Therapeutics Inc, Green Bay, Wisconsin 54311, USA; U — Digestive Support, Pet Naturals of Vermont, Essex Junction, Vermont 05453, USA; V — Probiotic Pearls, Integrative Therapeutics Inc, Green Bay, Wisconsin 54311, USA; W — Pet Inoculant, Wysong Corporation, Midland, Michigan 48642-7779, USA; X — Total Biotics Powder, Ultra-Pet Products LLC, Laguna Hills, California 92653, USA; Y — Living Pet Probiotic, Living Streams Mission, Coeur D-Alene, Idaho 83815, USA.

[a] Total numbers of all organisms.

[b] Percentage of label claim calculated as actual concentration (CFU/g)/label claim (CFU/g) ×100. NA — Not available — Unable to calculate percentage since label did not contain an expected number of viable organisms.

used doses of 200 million to 500 billion CFU/d (6–9), levels that are unachievable with most of the tested products. While objective dosing information for most of the tested commercial products is lacking, it is reasonable to assume that a product with high levels of viable organisms is much more likely to have the potential for efficacy than a product with low levels, since even if a product contained potentially beneficial organisms, low doses would likely preclude any chance of a positive effect. The potential for different organisms to have probiotic effects in vivo is also dependent on various factors, including acid tolerance, bile tolerance, ability to adhere to the intestinal cells or intestinal mucus in the target animal species, and bacteriocin production (10). None of these were evaluated in this study and there is minimal information describing potentially useful in vitro or in vivo properties for most veterinary probiotics.

Two of the products that met or exceeded label claims had high numbers of *Bacillus* spp. Bacilli are sporeforming bacteria and bacterial spores are very tolerant of environmental effects and storage; therefore, it is not surprising that high levels could be present. One product contained *E. faecium*, and while not as tolerant as bacterial spores, enterococci are able to persist better than many other vegetative bacteria and this may contribute to survival during probiotic processing and storage. In contrast, bifidobacteria are quite sensitive to environmental stress and have been absent in some previously reported studies of commercial probiotics (3,4). One product, however, contained greater than the stated concentration of *B. animalis*. This product was in a vegetable oil-based chewable formulation, which perhaps contributed to its survival. It has also been observed that *B. animalis* subsp. *lactis* is more tolerant to environment

stress (11), but it is unclear if this is also true for the *B. animalis* strain present in the tested product.

While exceeding label claim of viable organisms is encouraging from a product quality standpoint, the relevance of the label claim should also be considered. For example, 1 product contained 3194% of claimed *Bacillus subtilis* and mixed lactic acid bacteria, yet the label claim was very low: 18.55×10^6 and 54.12×10^6 CFU per kilogram, respectively. Labeling contents per kilogram is questionable for a product that only contained a total of 227 g. Such labeling may be misleading when most other products provide label claims per gram or dose. Even though this product exceeded the label claim, the number of viable organisms provided per recommended dose (1/4 to 3 tsp) would be quite low and unlikely to be effective. This highlights the need for close scrutiny of labels. Another product provided organism numbers per pound, but recommended doses of 3 to 15 g.

Misleading labeling of *Bacillus coagulans* was found in 3 products, with identification of that organism as *Lactobacillus sporogenes* or *Lactospore sporogenes* on the label and a footnote indicating the real name. The reason that some manufacturers use this misleading designation is unclear and unfounded microbiologically since neither the *Lactospore* genus nor *Lactobacillus sporogenes* exists and *B. coagulans* is not a lactic acid bacterium. The authors suspect that this is for marketing purposes because of the increasing consumer knowledge of lactic acid bacteria such as *Lactobacillus* spp.

Spelling errors were surprisingly common, being present on the labels of 32% of products that listed bacterial species. While spelling errors do not necessarily indicate a poor quality product, they certainly raise concern about the knowledge or attention to detail, and do not provide much confidence in the quality of the product. Veterinary practitioners would certainly be reluctant to use a pharmaceutical product that misspelled its contents, and the same degree of scrutiny should be applied to probiotics.

A wide range of microorganisms were claimed to be present in these products. Specific testing to identify each organism was not performed. Because of the typical similarity between closely related organisms (*Lactobacillus* species), differentiation of individual organisms and clearly identifying numbers of different related species among mixed products would be difficult to do with confidence. For example, if varying concentrations of different lactobacilli were present in a product, it would be difficult to accurately determine the relative numbers, or even presence of each species. Therefore, it was decided to restrict analysis to the genus level. Considering there is no information indicating that some species are different or better than other species, it was thought that identification of all species would not contribute much to the study. Accordingly, it is possible that products claiming to have mixed populations of lactobacilli or bacilli may not have had the claimed degree of diversity. The only 2 products that were deemed acceptable both contained only a single species. There are conflicting views about whether single organisms or blends of organisms are better, and there are no objective data either way. In the absence of efficacy studies, potential efficacy differences between single and multiple-organism products are solely conjecture.

Ultimately, proper efficacy studies are required to objectively identify effective probiotic organisms and doses. In the absence of evidence of efficacy, veterinarians and consumers must demand evidence of at least proper product quality control and marketing. Continued pressure needs to be exerted on manufacturers of probiotics to properly identify contents and ensure that the organisms claimed on the label are actually present at the time the probiotic reaches a patient. In the absence of regulatory scrutiny, pressure by the marketplace may be the only way to improve the quality of commercial veterinary probiotics.

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